Supplementary Data

Materials and Methods

Cells

Monolayer adherent HEK293T cells, HEK293T cells overexpressing Rev ($Rev10^+$ cells) and HeLa MAGI cells (TZM-bl) (1, 2) were grown in Dulbecco's modified Eagle's Medium (DMEM). The T-lymphocyte cell lines Sup-T1 and H9 were grown in RPMI 1640 medium. Cells other than the $Rev10^+$ cells were provided by the NIH Reagent Program, Division of AIDS, NIAID, NIH, Bethesda, MD, USA. Cells were incubated at 37°C in a 5% CO_2 atmosphere. All media were supplemented with 10% (v/v) fetal calf serum, 0.3 g/l L-glutamine, 100 U/ml penicillin and 100 U/ml streptomycin (Biological Industries, Beit Haemek, Israel). HeLaP4/shp75Cl15 cells (LEDGF/P75 knockdown cell line), a generous gift of Prof. Z. Debyser (Molecular Medicine, K.U. Leuven, Flanders, Belgium), were grown as described in (3). $Rev10^+$ and LEDGF/p75-knockdown Rev-expressing cells were generated by transfection into HEK293T and HeLaP4/shp75Cl15 cells, respectively (4) with pcDNA3.1 plasmid bearing the full Rev.

Viruses

WT HIV-1 (HXB2 (5)) and ΔEnv (6), as well as the IN mutant D64N D116N (7), were generated by transfection into HEK293T cells (4) of the virus-containing plasmid or co-transfected with a plasmid containing VSV-G (8). ΔRev pLAIY47H2 (9) and Rev M10 (10) HIVs were generated by transfection into $Rev10^+$ cells. Viruses were harvested and stored as described in (8). The pLAIY47H2 (9) viruses were a generous gift from Prof. B. Berkhout (Department of Human Retrovirology,

Academic Medical Center, University of Amsterdam, The Netherlands), and the IN mutant D64N D116N virus was a generous gift from Prof. A. Engelman (Department of Cancer Immunology and AIDS Dana-Farber Cancer Institute and Division of AIDS, Harvard Medical School, Boston, MA, USA).

Infection of cultured cells

Cultured lymphocytes were infected exactly as described in (8). Briefly, Cultured lymphocytes (1 x 10^5) were centrifuged for 5 min at 2000 rpm and after removal of the supernatant, the cells were resuspended in 0.2 to 0.5 ml of RPMI 1640 medium containing virus at the indicated multiplicity of infection (MOI). Following absorption for 2 h at 37^{o}C, the cells were washed to remove unbound virus and then incubated at the same temperature in RPMI 1640 medium. Cultured HEK293T cells, Rev10$^+$ cells and HeLa MAGI cells (TZM-bl) were grown for 24 h before infection, then the medium was discarded and cells were incubated at different multiplicity of infections (MOI) with the indicated virus for 2 h at 37^{o}C. Cells were washed three times with PBS and incubated in DMEM.

Virus stock titration and normalization

Quantitative titration of HIV-1 was carried out using the MAGI assay, as described by Kimpton and Emerman (2). Briefly, TZM-b1 cells were grown in 96-well plates at 1 x 10^4 cells per well. the cells were infected with 50 μl of serially diluted virus (wild-type, ΔRev or Rev M10 HIV-1) as described (2). Two days post-infection (PI), cultured cells were fixed and β-galactosidase was estimated exactly as described previously (2). Blue cells were counted under a light microscope at 200X magnification. In the case of IN mutant viruses (D64N D116N) the amount of viral

RNA was estimated by real time revers transcription PCR as described in (11). This was also proformed to the other HIV-1 viruses and was used to normalize the titer of the IN mutant virus.

Peptide synthesis and purification

Peptides were synthesized on an Applied Biosystems (ABI) 433A peptide synthesizer and purification was performed on a Gilson HPLC using a reverse-phase C8 semi-preparative column (ACE, Advanced Chromatography Technologies, USA) as described in (8).

Protein expression and purification

Expression and purification of histidine-tagged Rev-GFP conjugate was performed as previously described (12). The histidine-tagged IN and LEDGF/p75 expression vectors were a generous gift from Prof. A. Engelman and their expression and purification were performed essentially as described previously (13, 14). GST-Tat was expressed and purified as described previously (15).

ELISA-based binding assays

Protein-peptide, protein-protein and protein-DNA binding was estimated using an ELISA-based binding assay exactly as described previously (16). Briefly, Maxisorp plates (Nunc) were incubated at room temperature for 2 h with 200 ml of 10 μg/ml synthetic peptide/recombinant protein in carbonate buffer (0.05 M Na_2CO_3/0.05 M $NaHCO_3$, pH 9.6). After incubation, the solution was removed, the plates were washed three times with PBS, and 200 μl of 10% BSA (Sigma) in PBS (w/v) was added for 2h at room temperature. After rewashing with PBS, tested BSA- biotinilated

(Bb), peptide or protein (alone or biotinilated) or biotinilated DNA were added for further incubation for 1h at room temperature. Following three washes with PBS, the concentration of bound molecules was estimated after the addition of streptavidin-horseradish peroxidase (HRP) conjugate (Sigma), as described previously (17), or of anti-GFP mouse antibody (Santa Cruz) followed by rabbit anti-mouse IgG antibody conjugated to HRP. The enzymatic activity of HRP was estimated by monitoring the product's optical density (OD) at 490 nm using an ELISA plate reader (Tecan Sunrise Swizerland). Each measurement was performed in duplicate. For dissociation from and binding to a complex after binding of the first protein to the Maxisorp plate, the binding partner was incubated for 1 h at room temperature and after three washes with PBS, the dissociated component was added and its binding to the complex, as well the amount of remaining bound complex, were estimated separately as described above.

***In-vitro* IN activity assay**

Quantitative determination of IN activity was performed exactly as described previously (18) using a previously described assay system (19, 20). Briefly, the oligonucleotide substrate consisted of one oligo (5′-ACTGCTAGAGATTTTCCACACTGACTAAAAGGGTC-3') labeled with biotin at the 3′ end and the other oligo (5′-GACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGT-3') labeled with digoxigenin at the 5′ end. When inhibition was studied, the IN was preincubated with the peptide or protein for 15 min prior to addition of the DNA substrate. The entire IN reaction was followed by immunosorbent assay on avidin-coated plates as described previously (18, 20).

Plasmid construction

All of the plasmids used in this study were constructed using PCR cloning techniques with the highfidelity enzyme Platinum *Pfx* DNA polymerase (Invitrogen). Clones were subjected to automated DNA sequencing. For the bimolecular fluorescence complementation (BiFC) experiments, the yeast multicopy shuttle vectors pRS423 (with *HIS3* as the selective marker) and pRS426 (with *URA3* as the selective marker), both with the ADH1 promoter, were used as the cloning plasmids (a kind gift from Dr. D. Engelberg, Alexander Silberman Institute, The Hebrew University of Jerusalem). The DNA-coding region of the two yeast green fluorescent protein (GFP) fragments (21), namely the N terminus (GN) containing GFP amino acids 1–154, and theCterminus (GC) containing GFP amino acids 155–239, were cloned into pRS423 and pRS426, respectively. A linker consisting of $(GGS)_5$ was used to separate the inserted genes. The final vectors were termed GN-linker (cloned into pRS423) and GC-linker (cloned into pRS426). The coding sequences of full-length HIV-1 IN, Rev, LEDGF/p75 and Tat were individually amplified by PCR and inserted in-frame into the corresponding sites of the GN-linker and GC-linker to allow reunition of the two GFP halves into a functional protein upon protein-protein interaction as described in (18).

Bimolecular fluorescence complementation (BiFC)

The above-described plasmids were transformed into the yeast strain EGY48 (Clontech) and the cells were grown on yeast nitrogen base medium lacking histidine and uracil. After 48 h at 30 °C, the plates were transferred to 23 °C for 2 to 3 days and the appearance of fluorescence in yeast cells was visualized by confocal microscope

(MRC 1024 confocal imaging system, Bio-Rad), were as previously described (22) (18).

Study of *in-vivo* protein-protein interactions by co-IP

The co-IP experiments were conducted essentially as described previously (23) with several modifications. Briefly, cells were infected with a MOI of 15 for the indicated viruses. Cells were harvested at different times post infection (PI), washed three times in PBS and lysed by the addition of PBS containing 1% (v/v) Triton X-100 for whole-cell lysate. Cytoplasmic, nuclei and PIC fractions were isolated as described below. Half of the lysate or the isolated fraction was subjected to SDS-PAGE and immunoblotted with either a monoclonal anti-Rev antibody (α-Rev) (24) or antiserum raised against IN amino acids 276-288 (α-IN) (NIH AIDS Research & Reference Reagent Program catalog number 758), or anti-LEDGF/p75 (α-LEDGF/p75) (R&D Systems) or anti-actin (α-actin) antibody (Santa Cruz), and the complementary HRP-conjugated secondary antibodies (Jackson).

The remaining lysate or isolated fractions were incubated for 1 h at 4°C with either the α-Rev, α-IN, α-LEDGF/p75 or α-actin antibodies. Following a 3-h incubation with protein G-agarose beads (Santa Cruz) at 4°C, the samples were washed three times with PBS containing 1% (v/v) Nonidet P-40. SDS buffer was added to the samples and after boiling and subjecting to SDS-PAGE, the membranes were immunoblotted with either α-Rev, α-IN, α-LEDGF/p75 or α-actin antibodies, and the complementary HRP-conjugated secondary antibodies.

When peptides were used, cells were incubated with 150 μM of the indicated peptide for 2 h prior to infection.

Quantitative estimation of the bands was performed by Image Gauge V3.46 software (Fujifilm).

Isolation of cytoplasm, nuclei and PIC from infected cells

The various fractions were obtained from virus-infected cells essentially as described previously (25) with several modifications. Briefly, cells were harvested and washed twice in buffer A (20 mM Hepes pH 7.3, 150 mM KCl, 5 mM $MgCl_2$, 1 mM DTT and 0.1 mM PMSF). Cells were then suspended in 200 µl of buffer A with 0.025% (w/v) digitonin and incubated at room temperature for 10 min. Cells were centrifuged for 3 min at 1000*g* at room temperature. The supernatant was then centrifuged at 8000*g* and separated into supernatant (Cytoplasmic fraction) and pellet (nuclei fraction) and stored at -70^{o}C. For PIC isolation, an equal volume of buffer B (20 mM Hepes pH 7.4, 5 mM $MgCl_2$, 1 mM DTT and 0.1 mM PMSF) was added to the cytoplasm fraction. Samples were incubated for 10 min at room temperature and then centrifuged for 10 min at 2000*g*. The supernatant was discarded and the pellet, containing the PIC aggregates, was stored at -70^{o}C.

Cytoplasm, nuclei and PIC fraction Analysis

Cytoplasm and nuclei fractions were analyzed by western blot as described above. For detection of fraction specific protein ant actin antibody (Santa Cruz) and anti histone H3 antibody (abcam) were used.

For the anlysis of the PIC a total viral DNA was estimated by real time PCR as described below as well as integration of the PIC fraction *in-vitro* as described at (26).

Quantitative analysis of copy numbers of HIV-1 DNA integrated into the cellular genome (integration events)

The integration reaction, as well as the integration events, were performed exactly as described previously (8). Briefly, Integrated HIV-1 sequences were amplified by two PCR replication steps using the HIV-1 LTR-specific primer (LTR-TAG-F 5'-ATGCCACGTAAGCGAAACTCTGGCTAACTAGGGAACCCACTG-3') and Alu-targeting primers (first-Alu-F 5'-AGCCTCCCGAGTAGCTGGGA-3' and first-Alu-R 5'-TTACAGGCATGAGCCACCG-3') (27). Alu-LTR fragments were amplified from 10 ng of total cell DNA in a 25-µl reaction mixture containing 1X PCR buffer, 3.5 mM $MgCl_2$, 200 µM dNTPs, 300 nM primers, and 0.025 units/µl of *Taq* polymerase. The first-round PCR cycle conditions were as follows: a DNA denaturation and polymerase activation step of 10 min at 95°C and then 12 cycles of amplification (95°C for 15 s, 60°C for 30 s, 72°C for 5 min).

During the second-round PCR, the first-round PCR product could be specifically amplified by using the tag-specific primer (tag-F 5'-ATGCCACGTAAGCGAAACTC-3') and the LTR primer (LTR-R 5'-AGGCAAGCTTTATTGAGGCTTAAG-3') designed by PrimerExpress (Applied Biosystems) using default settings. The second-round PCR was performed on 1/25th of the first-round PCR product in a mixture containing 300 nM of each primer, 12.5 µl of 2X SYBR Green master mixture (Applied Biosystems) at a final volume of 25 µl, run on an ABI PRIZM 7700 (Applied Biosystems). The second-round PCR cycles began with DNA denaturation and a polymerase-activation step (95°C for 10 min), followed by 40 cycles of amplification (95°C for 15 s, 60°C for 60 s).

For generation of a standard calibration curve, the SVC21 plasmid containing the full-length HIV-1_{HXB2} viral DNA was used as a template. In the first-round PCR, the

LTR-TAG-F and LTR-R primers were used and the second-round PCR was performed using the tag-F and LTR-R primers. The standard linear curve was in the range of 5 ng to 0.25 fg (R = 0.99). DNA samples were assayed with quadruplets of each sample. For further experimental details see ref (18). The cell equivalents in the sample DNA were calculated based on amplification of the 18S gene by real-time PCR as described in (28).

Quantitation of total viral DNA

Total viral DNA was estimated using SYBR green real-time quantitative PCR 12 h PI, exactly as described in (29). Briefly, DNA samples (1 µg of DNA) were added to 95 µl containing 1×Hot-Rescue Real Time PCR Kit-SG (Diatheva s.r.l, Fano, Italy), and 100 nM of each PBS (primer-binding site) primer: F5 (5′ primer, 5′-TAGCAGTGGCGCCCGA -3′) and R5 (3′ primer, 5'-TCTCTCTCCTTCTAGCCTCCGC -3'). All amplification reactions were carried out using an ABI Prism 7700 Sequence Detection System (Applied Biosystems): One cycle at 95 °C for 10 min, followed by 45 cycles of 15 s at 95 °C and 35 s at 68 °C. In each PCR run, three replicates were performed.

Quantitative estimation of HIV-1 infection by determination of extracellular p24

The amount of p24 protein was estimated in the cell medium using an ELISA based capture assay kit (SAIC, AIDS Vaccine Program, Frederick, MD), according to the manufacturer's instructions and as described previously (18).

Immunostaining

HeLaP4/shp75Cl15 cells were grown on chamber slides (Nunc), then infected with ΔRev HIV-1 at a MOI of 25. Cells were fixed 16 h PI exactly as described previously (30) and immunostained essentially as described previously (30) with some modifications. Briefly, after fixation, cells were blocked with 5% IgG-free BSA (Jackson) in PBS for 60 min. For detection of HIV-1 IN and Rev and the host LEDGF/p75, the cells were incubated with 1:50 rabbit α-IN (NIH AIDS Research & Reference Reagent Program catalog number 758), 1:50 rat α-Rev (24) and 1:100 goat α-LEDGF/p75 (R&D Systems) at room temperature for 60 min each. Cells were washed five times with PBS + 0.05% (v/v) Tween 20 between antibodies. Then the cells were incubated with the following secondary antibodies: Cy2-conjugated anti-rat, Cy3-conjugated anti-rabbit and Cy5-conjugated anti-goat (Jackson) (all diluted 1:100) at room temperature for 60 min each, with five washes with PBS + 0.05% Tween 20 between antibodies. For detection of DNA, cells were stained with DAPI according to the manufacturer's protocol. Slides were prepared with Mounting Media (Bio-Rad) and immunofluorescent cells were detected with an Olympus confocal microscope.

Statistic analysis

$p < 0.05$, calculated from at least 3 repetitions for Real time analysis $p < 0.01$, ± stand for standard deviation.

Results

Characterization of the domains mediating the Rev-LEDGF/p75 interaction

We used a Rev-derived peptide library (AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: HIV-1 Consensus B Rev (15-mer) Peptides - Complete Set) to determine the sequences in Rev that specifically interact with the LEDGF/p75 protein. Two peptides, bearing residues 35 to 50 and 75 to 84 of the Rev protein, were found to interact with LEDGF/p75 (Figure S1A and H). Rev 35-50 and Rev 75-84 bear the Rev arginine-rich motif (ARM) and nuclear export signal (NES), respectively (31).

In addition, two LEDGF/p75-derived peptides (LEDGF 361-370 and LEDGF 402-411), which were previously found by us to interact with IN (32), also interacted with Rev-GFP (Figure S1B and H). It may be suggested that the peptides Rev 35-50, Rev 75-84, LEDGF 361-370 and LEDGF 402-411 represent the binding domains between Rev and LEDGF/p75. Support for this view was obtained from the results depicted in Figure S1C-F, showing that both groups of peptides were able to compete for binding and promote dissociation of the Rev-LEDGF/p75 interaction. Moreover, a mutual interaction between these two groups of peptides was observed (Figure S1G and H).

IN enzymatic activity of PIC isolated from HIV-1 infected cells.

The results in Figure S2A and B show that the PIC fraction obtained from a wt infected cells bears-as expected-viral cDNA as well as an enzymatic active IN. Optimal activity was observed in PIC obtained from infected cell between 10-16h PI. Both viral cDNA and IN enzymatic activity were diminished in PIC obtained from cells after 20h PI probably due to intracellular degradation processes. On the other hand in PIC isolated from infected cells with over expressing Rev, the amount of

cDNA was the same as that observed as in wt cells but no IN enzymatic activity was detected due probably to the Rev inhibitory effect.

Characterization of the Cytoplasm and Nuclei fraction of HIV infected cells.

Our western blot analysis clearly has indicated that no cross contamination has been occurred between the cytoplasm and nuclei fraction obtained from viral infected cells. This is evident from the results showing the presence of histone H3 protein in the nuclei but not in the cytoplasm fraction and on the other hand the presence of actin molecules in the cytoplasm but not in the nuclei fraction (Figure S2C).

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Figure S1: Identification and characterization of Rev-LEDGF/p75 interacting domains.

(A) LEDGF/p75 protein was added, in increasing amounts, to the following plate-bound peptides: Rev 13-23 (□), Rev 35-50 (▲), Rev 53-67 (O) and Rev 75-84 (♦) and the amount of bound LEDGF/p75 was estimated. **(B)** Rev-GFP and GFP proteins were added to plate-bound LEDGF 361-370 (●,◊ respectively) and LEDGF 402-411 (■,Δ respectively) peptides and the amount of Rev or GFP bound proteins was estimated. **(C)** Increasing amounts of the following peptides: LEDGF 361-370 (●), LEDGF 402-411 (■) and INr-1 (IN 66-80) (8) (Δ, control), were mixed with 100 nM LEDGF/p75 and the binding of LEDGF/p75 to plate-bound Rev-GFP was estimated. **(D)** Increasing amounts of the following peptides: Rev 35-50 (▲), Rev 75-84 (♦) and Rev 13-23 (18) (□, control), were mixed with 100 nM Rev-GFP and the binding of Rev-GFP to plate-bound LEDGF/p75 was estimated **(E)**. Same as in **(C)** except that the peptides were added to an already plate-bound Rev(-GFP)-LEDGF/p75 complex. **(F)** Same as in **(D)** except that the peptides were added to an already plate-bound Rev(-GFP)-LEDGF/p75 complex. **(G)** Binding of Rev 35-50 peptide to plate-bound LEDGF 361-370 (▲) and LEDGF 402-411 (■) peptides, and binding of Rev 75-84 peptide to plate-bound LEDGF 361-370 (●) and LEDGF 402-411 (♦) peptides. **(H)** Apparent K_d as calculated according to the results obtained in **A, B** and **G.** All other details are described in Materials and methods.

Figure S2: Cytoplasm, Nuclei and PIC fraction analysis

The different cells fractions of HIV-1 infected wt and over expressing Rev cells were isolated as described in Material and Methods and Figure 3 at different times after infection. The amount of viral cDNA (A) and the viral IN enzymatic activity (B) were estimated by real time PCR as described in Material and Methods. Western blot

analysis (C) for detection of actin and histone H3 was performed as described in Materials and Methods.

Table legend

Supplementary Table 1

Quantitative estimation of the co-immunoprecipitation (co-IP) (see Figure 3) bands was performed by Image Gauge V3.46 software (Fujifilm)

Supplementary Table 1A

Time	Fraction	IP with	Anti-Rev	Anti-IN	Anti-LEDGF
6 h PI	Nucleus	Actin	0.51	0	1.06
		Rev	0.16	0	0
		IN	0.81	5.98	22.82
		LEDGF	0.69	5.94	41.93
	Cytoplasm	Actin	0.31	0	0
		Rev	28.37	5.85	12.96
		IN	15.66	30.35	16.02
		LEDGF	2.57	3.72	26.2
	PIC	Actin	1.77	0	0
		Rev	3.89	0.98	1.27
		IN	5.22	32.83	16.15
		LEDGF	1.97	11.81	26.49
10 hrs PI	Nucleus	Actin	1.63	0	0.02
		Rev	54.67	6.03	24.08
		IN	26.56	31.26	42.07
		LEDGF	27.79	36.53	49.1
	Cytoplasm	Actin	1.59	0.02	0.14
		Rev	56.08	31.13	14.96
		IN	57.3	30.42	16.48
		LEDGF	20.83	7.23	28.77
	PIC	Actin	1.11	0	0
		Rev	34.47	7.09	17.7
		IN	29.51	31.77	15.87
		LEDGF	12.48	34.09	25.06
16 h PI	Nucleus	Actin	6.08	0	0.11
		Rev	68.14	37.09	47.01
		IN	65.88	32.12	42.87
		LEDGF	66.91	31.93	41.43
	Cytoplasm	Actin	6.67	3.25	5.23
		Rev	59.36	27.46	15.82
		IN	68.39	32.79	11.57
		LEDGF	23.18	8.81	12.37
	PIC	Actin	8.4	0.06	0.44
		Rev	10.41	2.78	3.07
		IN	8.12	2.45	3.02
		LEDGF	8.59	2.51	2.91
20 h PI	Nucleus	Actin	6.25	0.24	0.34
		Rev	68.64	0.11	39.76
		IN	65.5	5.98	14.38
		LEDGF	64.85	32.42	41.6
	Cytoplasm	Actin	7.48	30.82	2.47
		Rev	65.05	29.68	10.14
		IN	63.06	33.18	4.26

		LEDGF	29.89	3.88	18.72
	PIC	Actin	7.24	0.06	1.33
		Rev	8.14	0	0
		IN	9.03	0	0
		LEDGF	6.72	0	0

Supplementary Table 1B

Times	IP with:	Anti-Rev	Anti-IN	Anti-LEDGF	Anti-Actin
6 h PI	Lysate	81.54	10.82	56.75	30.4
	Actin	0.38	8	0.11	36.53
	Rev	81.28	11.13	58.53	0
	IN	19.62	11.04	0.07	0
	LEDGF	80.19	8	54.67	0
10 h PI	Lysate	73.66	10.71	50.14	30.23
	Actin	0.36	8	0.03	35.3
	Rev	83.29	11.3	60.31	0
	IN	21.54	11.26	0.03	0
	LEDGF	83.2	8	60.28	0
16 h PI	Lysate	78.48	21.74	56.76	32.83
	Actin	0.79	8	0	38.12
	Rev	77.82	20.94	53	0.09
	IN	36.82	21.39	0	0
	LEDGF	80.61	8	57.83	0
20 h PI	Lysate	81.65	22.29	56.97	32.8
	Actin	1.26	8	0	36.57
	Rev	80.72	22.22	59.18	0
	IN	40.87	22.58	0.02	0
	LEDGF	91.07	8.01	64.68	0

Supplementary Table 1C

Times	IP with:	Anti-Rev	Anti-IN	Anti-LEDGF	Anti-Actin
6 h PI	Lysate	0.24	19.2	53	44.75
	Actin	0.25	7	0.15	47.47
	Rev	0.25	7	0	0.22
	IN	0.25	20.89	11.08	0
	LEDGF	0.26	20.82	56.39	0
10 h PI	Lysate	0.26	35.17	52.96	40.66
	Actin	0.26	7	0	45.36
	Rev	0.26	7	0	0.23
	IN	0.26	40.35	28.13	0
	LEDGF	0.24	40.25	61.34	0
16 h PI	Lysate	0.24	77.95	57.47	42.78
	Actin	0.24	7	0	48.8
	Rev	0.25	7	0.03	0.95
	IN	0.26	79.73	57.77	0
	LEDGF	0.26	80.29	59.35	0
20 h PI	Lysate	0.25	80.86	60.75	43.68
	Actin	0.25	7	0	44.36
	Rev	0.25	7.02	0	0.71
	IN	0.26	81.83	62	0
	LEDGF	0.25	88.43	67.87	0

Supplementary Table 1D

Times	IP with:	Anti-Rev	Anti-IN	Anti-LEDGF	Anti-Actin
6 h PI	Lysate	17.82	10.37	0.24	23.62
	Actin	0.01	0	0.28	29.1
	Rev	17.59	11.95	0.27	0.11
	IN	17.2	11.34	0.28	0
	LEDGF	0	0	0.27	0
10 h PI	Lysate	58.52	10.28	0.28	24.82
	Actin	0	0	0.28	27.71
	Rev	69.27	12.46	0.27	0.01
	IN	19.55	12.31	0.28	0
	LEDGF	0	0	0.28	0
16 h PI	Lysate	64.1	11.44	0.28	25.78
	Actin	0	0	0.27	30.13
	Rev	62.33	11.08	0.28	0.03
	IN	17.18	11.21	0.28	0
	LEDGF	0.01	0	0.28	0
20 h PI	Lysate	66.58	11.88	0.28	27.04
	Actin	0	0	0.28	28.74
	Rev	66.81	11.72	0.28	0.01
	IN	19.99	12.23	0.27	0
	LEDGF	0	0	0.27	0

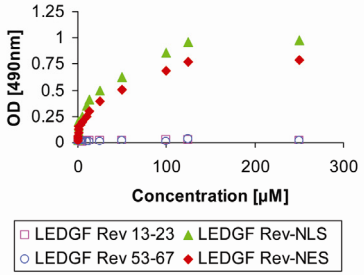
Supplementary Table 1E

Times	IP with:	Anti-Rev	Anti-IN	Anti-LEDGF	Anti-Actin
6 h PI	Lysate	0.37	10.3	0.46	30.97
	Actin	0.37	0	0.48	33.59
	Rev	0.37	0.04	0.48	0.01
	IN	0.38	11.19	0.49	0
	LEDGF	0.41	0	0.48	0
10 h PI	Lysate	0.38	10.36	0.46	29.43
	Actin	0.43	0	0.55	31.89
	Rev	0.43	0	0.56	0.18
	IN	0.42	12.14	0.52	0
	LEDGF	0.44	0	0.56	0
16 h PI	Lysate	0.39	11.42	0.50	29.54
	Actin	0.38	0	0.49	32.69
	Rev	0.38	0	0.48	2.1
	IN	0.37	10.99	0.49	0
	LEDGF	0.38	0	0.50	0
20 h PI	Lysate	0.38	12.08	0.56	30.39
	Actin	0.38	0	0.56	29.63
	Rev	0.40	0	0.55	2.9
	IN	0.41	12.21	0.55	0
	LEDGF	0.40	0	0.56	0

Supplementary Table 1F

Times	Peptide	IP with:	Anti-Rev	Anti-IN	Anti-LEDGF
6 h PI	INr-1 & 2	Rev	26.42	0.01	7.86
		IN	0.83	17.02	19.95
		LEDGF	0	17.44	42.58
	Rev 13-23 & 53-67	Rev	18.08	0	6.48
		IN	0	16.68	21.52
		LEDGF	0	15.04	46.15
	Rev 35-50 & 75-84	Rev	91.13	6.6	0.04
		IN	24.39	39.75	21.74
		LEDGF	0	16.12	52.39
	LEDGF 361-370 & 402-411	Rev	92.76	6.73	0
		IN	19.41	38.63	0.36
		LEDGF	0.02	0.01	52.63
10 h PI	INr-1 & 2	Rev	16.46	1.3	19.81
		IN	0.02	41.06	46.03
		LEDGF	19.65	36.47	49.39
	Rev 13-23 & 53-67	Rev	23.6	0.02	20.75
		IN	0	41.59	46.67
		LEDGF	18.95	36.51	58.28
	Rev 35-50 & 75-84	Rev	111.49	39.95	0.02
		IN	97.09	41.2	50.18
		LEDGF	0.01	40.46	55.05
	LEDGF 361-370 & 402-411	Rev	108.23	41.8	0.05
		IN	109.81	35.24	0.02
		LEDGF	0	0	51.15
16 h PI	INr-1 & 2	Rev	18.23	0	47.29
		IN	0.01	30.04	12.37
		LEDGF	85.94	11.41	50.78
	Rev 13-23 & 53-67	Rev	18.75	0.03	51.58
		IN	0.01	31.07	12.14
		LEDGF	83.08	11.79	40.16
	Rev 35-50 & 75-84	Rev	90.12	29.11	0.93
		IN	83.24	35.53	31.41
		LEDGF	0.01	10.23	53.27
	LEDGF 361-370 & 402-411	Rev	91.76	32.44	0
		IN	86.63	33.35	0.3
		LEDGF	0	0	52.81
20 h PI	INr-1 & 2	Rev	15.81	0	45.57
		IN	0	28.8	12.26
		LEDGF	86.09	10.08	50.96
	Rev 13-23 & 53-67	Rev	17.18	0	56.01
		IN	0	31.7	15.35
		LEDGF	90.36	12.14	53.33
	Rev 35-50 & 75-84	Rev	80.62	31.64	0.01
		IN	77.85	34.89	34.09
		LEDGF	1.09	11.33	50.3
	LEDGF 361-370 & 402-411	Rev	92.01	32.36	0
		IN	86.65	38.89	0
		LEDGF	0	0.01	55.22

A



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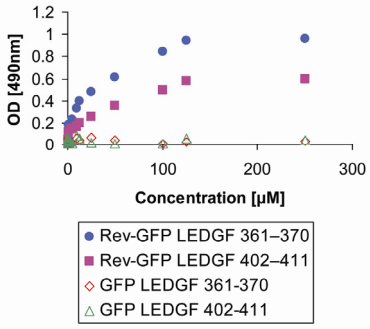

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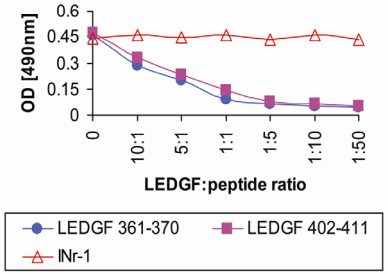

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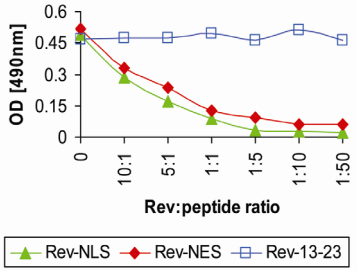

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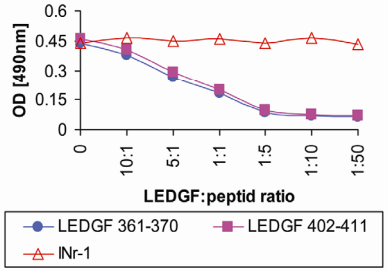

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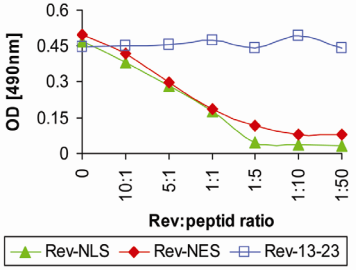

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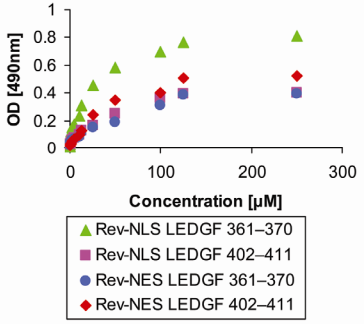
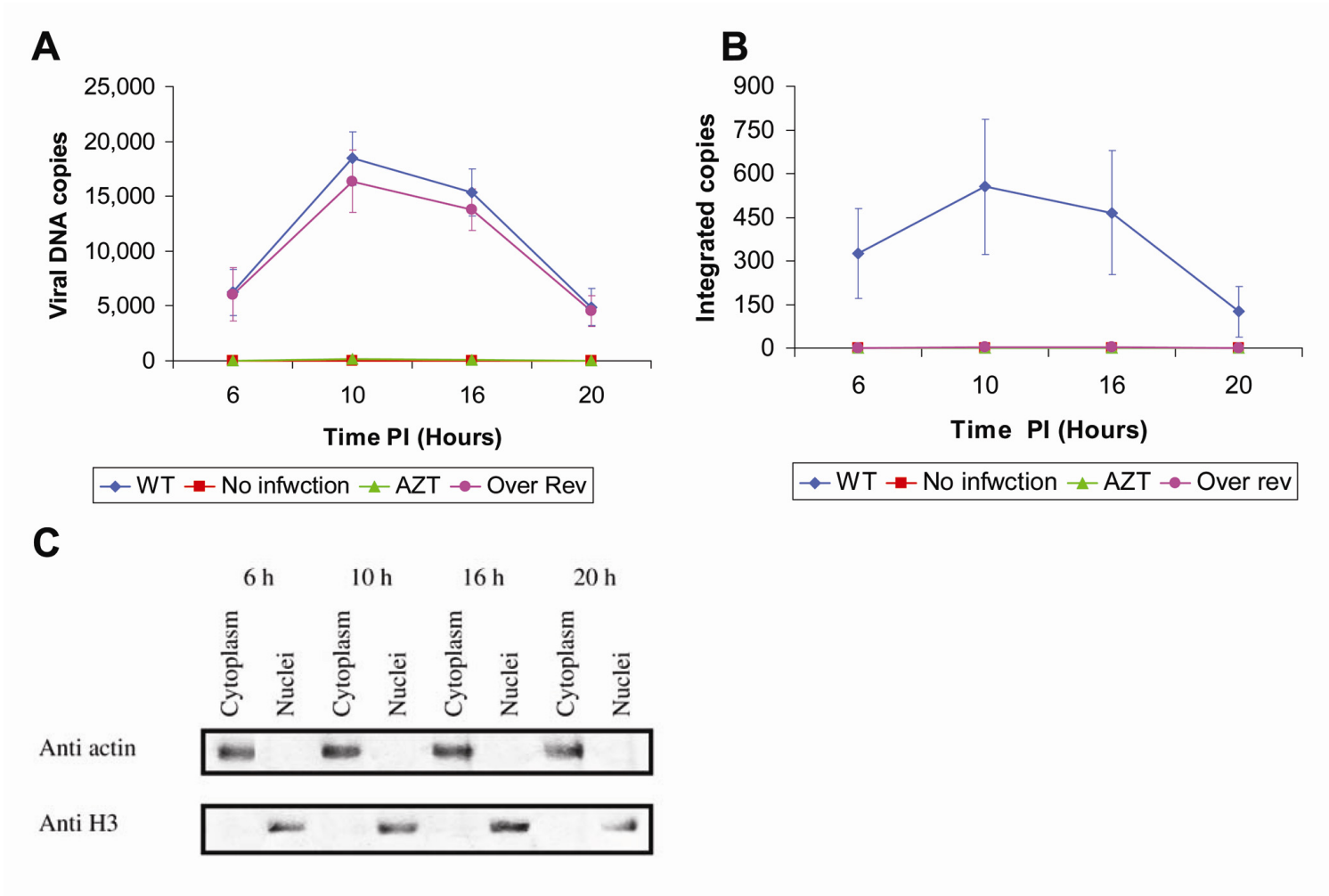


H

Name	Binding to	Apparent K_d [µM]
Rev 35-50	LEDGF	10.2 ± 1.8
	LEDGF 361-370	10.1 ± 1.7
	LEDGF 402-411	17.5 ± 1.2
Rev 75-84	LEDGF	13.6 ± 2.4
	LEDGF 361-370	29.6 ± 3.3
	LEDGF 402-411	16.3 ± 3.4
LEDGF 361-370	Rev-GFP	10.3 ± 2.7
LEDGF 402-411	Rev-GFP	18.3 ± 1.4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10
16
20
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No infwction
AZT
Over Rev
B
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750
600
450
300
150
0
Integrated copies
6
10
16
20
Time PI (Hours)
WT
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AZT
Over rev
C
6 h
10 h
16 h
20 h
Cytoplasm
Nuclei
Cytoplasm
Nuclei
Cytoplasm
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Cytoplasm
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Anti actin
Anti H3